A Vertical Invasion Assay for Use With Stem Cell Fusion Products

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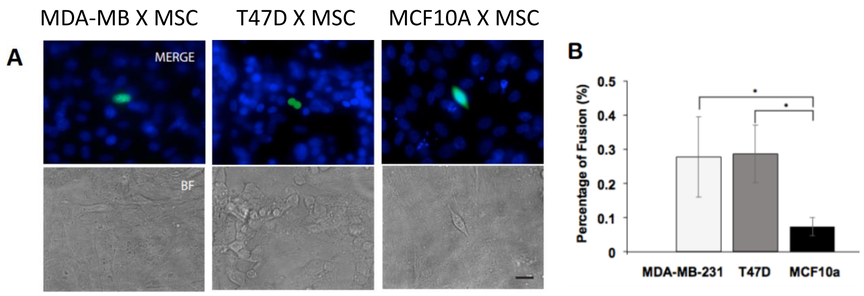
Abstract

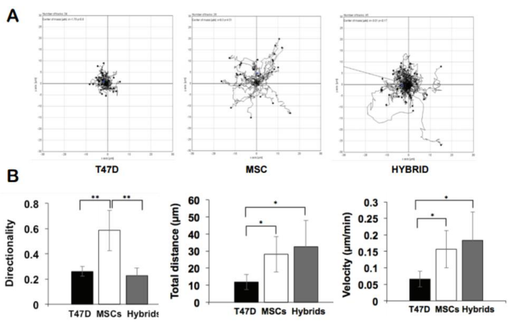
Metastasis is the leading cause of death among cancer patients. Thus, understanding the mechanisms through which metastasis occurs is a very important area of research. In order to metastasize, cancer cells should be able to move from their primary site to other locations and repopulate those sites. Our hypothesis is that primary cancer cells fuse with cells that migrate thereby enabling the hybrid to disseminate and divide. To test our hypothesis *in vitro,* we are developing a vertical invasion assay capable of assessing the invasion capabilities of fusion products in a three dimensional environment.[1] Our preliminary data show that mesenchymal stem cells, which are one of the main fusion partners of cancer cells, can migrate vertically into the three dimensional environment we are developing. This assay will aid in analyzing the metastatic capability of both cancerous cells and associated fusion hybrids and help understand better the mechanisms of metastasis with the hope of developing new therapeutic targets to inhibit cancer cell fusion and metastatic spread.

Motivation: MSCs fuse spontaneously with breast epithelial cells (*)



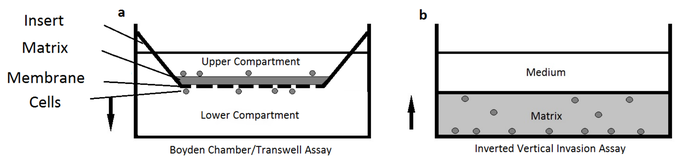
a: Representative fusion products from MSC x breast epithelial cell (MDA-MB and T47D) co-cultures. Scale bar, 25 µm. **b:** Frequency of fusion of MSCs with breast epithelial cells. Each value represents the average of 3 independent experiments done in triplicate.

Motivation: Hybrids between MSCs and T47D show higher migratory capability (*)



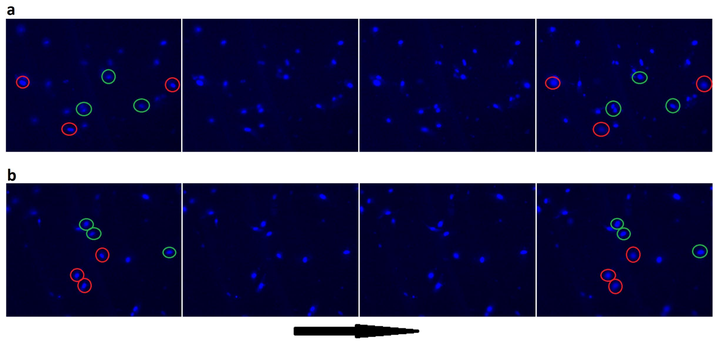
Migration of hybrid cells compared to that of MSCs alone or T47D alone (time-lapse microscopy). **a:** Representation of motility patterns of T47D, MSCs, and hybrids. **b:** Directionality of motion of cells, Total accumulated distance, and Velocity. Each value represents the average of four independent experiments with the analysis of at least 50 cells per group and pre experiment. ANOVA Tukey's HSD *post hoc* test (*P<0.05, **P<0.01, mean ± SD).

Current Design Allows Cells to Remain in Original Microenvironment



a) *Traditional Boyden Chamber or Transwell Invasion Assay.* This assay requires that the cells are collected from the original culture location and plated onto the transwell insert. The bottom of the insert is a porous membrane which allows for cell migration into the underlying medium. To measure invasion versus migration the porous membrane is coated with a layer of extracellular matrix protein. **b)** *Inverted Vertical Invasion Assay currently proposed*. This design allows the cells to remain within the original microenvironment. The layer of matrix protein is cast atop the cell layer. After polymerization, medium is overlaid atop the matrix layer. Confocal imaging is used to assess the number of cells invading into the collagen matrix.

Preliminary Experimentation Shows Potential of Cells to Invade Into Collagen Matrix



a-b) Z series of two locations within a dish following three days of the execution of the vertical invasion assay. Each successive image is 24µm above the previous. The images were taken on a preliminary trial of the assay, further optimization of the x, y, and z location of the images is ongoing. Red and green circles aid in the tracking of individual cells along the z axis. Green circles indicate putative invading cells; note, in focus (right-most image) invading into gel, out of focus (left-most image) beneath gel.

Methods and Materials

- *Treatment of MatTek Dishes:* Before plating cells, each 35 mm dish was washed with I M hydrochloric acid for fifteen minutes, then washed with PBS, 70% ethanol, and cell culture medium. The treatment ensures the hydrophobicity of the glass bottom wells is sufficiently low to allow the collagen gel to disperse evenly throughout the well.
- *Cells:* Each dish was plated with 50,000 mesenchymal stem cells.
- *Collagen Gel:* A collagen gel solution was created using rat tail collagen (3.8 mg/mL), 10x DMEM, and 1x α-MEM (20% FBS). An appropriate amount of 4.5% sodium bicarbonate was added to ensure that the solution was neutral. Eighty µL of collagen solution was plated within each 10mm MatTek well. After allowing thirty minutes for the gel to polymerize, 1x α-MEM (10% FBS) was overlaid atop the wells.
- *Fixing of the Assays:* On day one, two, and three the wells were fixed with 4% paraformaldehyde for 15 minutes. The fixed dishes were then stained with DAPI (100 ng/mL) for 20 minutes.
- *Imaging:* Z-stack images were taken at prescribed locations on each dish using a confocal microscope.

Conclusions

- The proposed vertical invasion assay may be useful to measure invasive capabilities of cancer cells and cancer cell fusion products.
- Further iterations could include various combinations of extracellular matrix proteins that may more accurately mimic those of the tumor microenvironment.
- Assay may be valuable to probe mechanism of cancer metastasis
- *Limitations:* Invasion was not uniform or consistent between assays, so may require a stronger chemoattractant in the collagen gel.
- Occasionally, collagen gel lifted from the well and so care is required when executing the assay.

Acknowledgements:

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References:

1. Hooper, Steven, John F. Marshall, and Erik Sahai. "Tumor Cell Migration in Three Dimensions." Methods in Enzymology 406 (2006): 625-43. ScienceDirect. Web. 29 Aug. 2014.